Speciation: **More than the sum of the parts**

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Genetic studies are beginning to provide insights into the evolutionary processes that reduce the fitness of hybrids between recently diverged species. However, the deleterious gene interactions responsible for this fitness reduction are still poorly understood.

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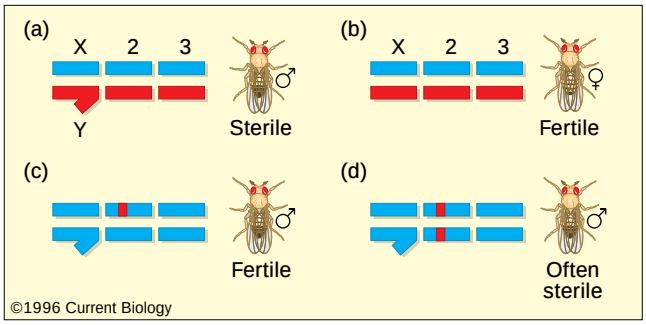
Organisms function through interactions between many thousands of genes and their products. Yet evolutionary biology has largely ignored such interactions: genes are seen as being selected for their individual effects, and quantitative traits are seen as being the sum of contributions from individual genes. There has long been dissatisfaction with this contrast between the largely additive framework of classical quantitative genetics, and the obvious prevalence of gene interactions in actual organisms. However, evidence of deviations from additivity — epistasis — is hard to come by for genes segregating within species [1]. Over the last decade, the greatest progress has come from study of the interactions that prevent gene exchange between species. The current flurry of work on the genetic basis of hybrid inviability and sterility promises to illuminate not only the nature and origin of species, but also the way genes combine to sustain normal function.

The genetic analysis of species differences requires that at least some hybrids be fertile. As one would expect from a gradual evolutionary process, such partial isolation is common. It is then possible to backcross first generation (F1) hybrids to one or other parent, and hence to determine the effects of particular chromosomes, or (with repeated backcrossing) of small chromosome regions. Such classical genetic techniques have been applied most extensively in *Drosophila*. They have revealed that, even between sibling species that are morphologically almost indistinguishable, sterility and inviability usually involve many genes. Even where a single gene of major effect is initially detected, higher-resolution mapping may resolve this into a cluster of linked loci with interacting effects [2]. For example, Davis and Wu [3] found that the middle 15 % of the X chromosome, which caused male sterility when introgressed from *Drosophila mauritiana* into *D. simulans*, contained at least six genetic factors.

Many species crosses show a particular kind of partial isolation, in which only one sex is infertile or inviable in the F1 hybrids. That sex is most often the one carrying distinct sex chromosomes — XY males in mammals and *Drosophila*, and ZW females in birds and Lepidoptera — a pattern known as 'Haldane's Rule' [4] (Fig. 1a,b). A related pattern is that, in conventional backcross analyses, the X chromosome tends to show a disproportionately large effect on hybrid fertility and viability [5]. Three explanations have been put forward for these observations. First, genes responsible for isolation may diverge faster on the X than on the autosomes. This would ensue if advantageous alleles tend to be recessive, and so are more easily picked up by selection if expressed in a single copy in the heterogametic sex [5]. Second, even if the underlying rates of evolution are the same, deleterious interactions involving recessive alleles on the X would be detected more easily in the heterogametic sex [6,7]. And last, Wu *et al.* [8] have argued that, in *Drosophila* and mammals, Haldane's Rule is largely due to the intrinsically faster evolution of male sterility, rather than an effect due to sex linkage *per se*. However, this does not explain Haldane's Rule in birds and butterflies, where the females are heterogametic, and does not account for the large effect of the X chromosome.

Controversy over the causes of Haldane's Rule has dominated recent work on the genetics of speciation. Three recent developments may help resolve this and wider issues. These are first, the discovery of fertile hybrids from the cross between *D. simulans* and *D. melanogaster* [9]. Second, the construction of introgressed lines that are

Figure 1



Examples of the genotypes involved in the genetic analysis of crosses between *D. mauritiana* and *D. simulans*. Blue bars represent *D. simulans* chromosomes, whereas red bars represent *D. mauritiana* chromosomes. The sex chromosomes and two autosomes are illustrated. **(a,b)** Haldane's Rule: F1 males are sterile (a), but F1 females are fertile (b). **(c,d)** Examples of the partially hybrid genotypes created by True *et al.* [11]: males heterozygous (c) or homozygous (d) for a small autosomal region from *D. mauritiana*, embedded in a *D. simulans* background; the former are fertile, but the latter often sterile.

homozygous for autosomal regions [10,11]. And third, the quantitative analysis of differences in genital shape between *Drosophila* sibling species [12]. As well as helping to resolve the particular questions of whether reproductive isolation is due to recessive alleles, and whether incompatibility genes evolve faster on the X chromosome than the autosomes, these developments also open the way to understanding the nature and causes of epistatic interactions between genes.

In the cross between *D. melanogaster* females and *D. simulans* males, sons die, whereas in the reciprocal cross, daughters die. Several 'rescue' genes have been found, however, each of which allows hybrids in one or other reciprocal cross to survive [13–15]. This is remarkable, because inviability in this cross had been thought to be due to genes on every chromosome [16], and yet it can apparently be suppressed by a single locus. Until now, all the surviving hybrids were thought to be sterile, but Davis *et al.* [9] recently found a strain of *D. simulans* that gives fertile female hybrids with *D. melanogaster*. This will allow the genetic tools developed with *D. melanogaster* to be used to unravel the genetics of the isolation of *D. melanogaster* from *D. simulans*.

Progress is possible even without the tricks available with *D. melanogaster*. To date, most comparisons have been between the effects on hybrids of hemizygous X chromosomes and heterozygous autosomes. These can only detect recessive alleles if they are X-linked and in the heterogametic sex. Two recent studies have made a more relevant comparison by constructing lines which carry a region homozygous for *D. mauritiana* (or *D. sechellia*) genes, embedded in a homozygous *D. simulans* background. Hollocher and Wu [10] did this by selecting for visible markers on the second chromosome, and so assaying the effects of three large overlapping regions. True *et al.* [11] made a systematic survey of the whole genome by randomly inserting a transposable P element, and then selecting for an eye-colour marker carried by the transposon through fifteen backcross generations. This generated replicate lines carrying an introgressed region, 9.4 cM long on average, in 87 different locations, which were tested for viability and fertility in both sexes and as both heterozygotes and homozygotes.

The results from both methods are consistent, though not conclusive. First, effects are largely recessive: almost all autosomal regions have no effect as heterozygotes. This strongly supports the dominance theory [6,7]. Second, the X chromosome and the autosomes diverge at comparable rates. Hollocher and Wu [10] found that sterility and inviability are caused by factors occurring at essentially the same frequency on the X chromosome and the autosomes. True *et al.* [11] found that 75 % of introgressions on the X chromosome cause male sterility, compared with 50 % of homozygous autosomal introgressions. Although this difference in rate appears insufficient to account for the large X-chromosome effect, the introgressed segments on the X chromosome may be smaller than those on the autosomes and so it remains possible that the X chromosome does accumulate sterility factors substantially faster. Third, both studies found that male sterility evolves much more rapidly than female sterility. Thus, True *et al.* [11] found that 5.4 % of homozygous autosomal segments caused female sterility, whereas 50% caused male sterility. This may in part explain Haldane's Rule for sterility in *Drosophila* and mammals, but of course cannot account for Haldane's Rule for inviability, or for the patterns in birds and Lepidoptera, where the females are heterogametic.

Most genetic analyses of speciation use a crude qualitative classification of 'fertility' or 'inviability'. In contrast, a more rigorous statistical approach has been taken to the mapping of loci responsible for quantitative traits, mainly in domesticated species. Liu *et al.* [12] have used the statistical procedures of quantitative genetics to dissect the differences in genital morphology that distinguish *D. simulans* and its sibling species, and that might impede mating between them. They found that eight of the fifteen genomic intervals analysed affected the trait, and that, in contrast to fertility and viability, these had largely additive effects. Further progress may require that this approach be extended to fertility and viability. For example, the dominance coefficient that is central to theoretical explanations of Haldane's Rule [7] could only be estimated from an experiment of the kind performed by True *et al.* [11] by using a quantitative assay for fitness; substantial heterozygous effects may be missed with a crude classification into, say, 'fertile' or 'sterile'. Haldane's Rule and the large X-chromosome effect apply to fitness traits, not to morphology or behaviour [3], but without the use of comparable quantitative methods, it will be impossible to know whether morphology and reproductive isolation evolve in essentially different ways.

What does all this tell us about the evolution of reproductive isolation in nature? There is a large gap between the crude tests used in laboratory genetics and the behavioural and ecological factors that separate actual species. Sadly, it is hardly feasible to measure fitness under natural conditions. However, encouragement comes from the experimental crosses between sunflower species carried out by Rieseberg *et al.* [17]. Here, instead of measuring fitness directly, as in the *Drosophila* work described here, the introgression of multiple markers was followed through many backcross generations. The pattern of introgression observed was similar to that in natural hybrid populations, showing that indirect estimates of fitness from artificial crosses do reflect fitness in nature [17,18]. The ready availability of molecular markers may allow the kind of detailed genetic analyses that at present are largely confined to

Drosophila to be extended to organisms more amenable to study in nature.

If diverging populations are geographically separated, or allopatric, then the process of divergence is not affected by the incidental reproductive isolation that it causes. If so, measurement of hybrid fitness in nature is not so crucial, as it does not actually affect the process of speciation. Moreover, the genetic differences between *Drosophila* sibling species are greater than is needed to establish full reproductive isolation, and it is impossible to know which gene combinations first took the diverging populations past the formal threshold of speciation. Hybrid breakdown can be seen as telling us how genes work together. By this view, understanding the deleterious gene interactions revealed in hybrids gives the same kind of information as do laboratory screens for 'synthetic lethals' — mutations that are lethal in combination but not alone (see [19], for example). The genetic basis of hybrid breakdown can also give indirect information about the causes of divergence. For example, if sterility and inviability factors tend to be linked, and if this is not because of chance or the clustering of functionally related or homologous genes, then this implies that the diverging alleles tend to be substituted simultaneously — alleles that work well together are more readily substituted if they are linked [2].

To understand how divergence might have occurred, and how it might affect gene exchange, we need to know the fitnesses of all the recombinant genotypes — not just the fitness of F1 hybrids, on which attention has centred thus far. This is a difficult problem, both theoretically and empirically. To see this, consider how the recessiveness of incompatibilities might be explained. Within species, deleterious mutations tend to be recessive because they cause a loss of function that can be restored by a single wild-type allele. It is appealing to argue by analogy that incompatibilities are inherently recessive because they reflect a loss of function against a foreign genetic background [5]. This explanation fails, however, if interactions are between particular pairs of genes (one autosomal, one X-linked, say). In F1 males, the X-linked allele from one species would still be able to combine successfully with its autosomal partner, derived from the same species — loss of function should not be complete.

For Haldane's Rule to work, an allele from one species must fail against a heterozygous genetic background when it is homozygous or hemizygous. This is harder to envisage biochemically, and also implies that autosomal segments should cause death or sterility when introgressed as heterozygotes, contrary to observation (B. Charlesworth, personal communication). What is needed is a plausible biochemical model that explains deleterious gene interactions, analogous to that for the recessiveness of deleterious mutations [20]. The work described here suggests that it will not be long before a combination of classical genetics with molecular markers will allow such models to be tested.

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